

Serial No.: 09/557,423
Filed: April 22, 2000



REMARKS

Claims 43-45 and 52-54 have been amended. Claim 113 has been added. Claims 43-45, 52-66, 108, and 112-113 are pending in the application.

Please direct any questions to the undersigned at (415) 781-1989.

Respectfully submitted,

FLEHR HOHBACH TEST
ALBRITTON & HERBERT LLP

Richard F. Trecartin
Reg. No. 31,801

4 Embarcadero Center
Suite 3400
San Francisco, CA 94111
(415) 781-1989

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

1. (Cancelled) A composition comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each comprising:
 - a) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and
 - b) at least one locking sequence.
2. (Cancelled) The composition of claim 1 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of said locking sequences.
3. (Cancelled) The composition of claim 1 wherein said locking sequences form a triplex anchor.
4. (Cancelled) The composition of claim 1 wherein said locking sequences form a quadruplex anchor.
5. (Cancelled) The composition of claim 1 wherein said locking sequences form a Z-DNA anchor.
7. (Cancelled) The composition of claim 1 wherein said locking sequences form an A-DNA anchor.
8. (Cancelled) The composition of claim 1 wherein said locking sequences comprise RNA.
9. (Cancelled) The composition of claim 1 wherein said locking sequences comprise DNA.
10. (Cancelled) The composition of claim 1 wherein at one of said targeting polynucleotides comprises a peptide nucleic acid.
11. (Cancelled) The composition of claim 1 wherein said locking sequences comprise DNA and RNA.
12. (Cancelled) The composition of claim 1, wherein said recombinase is a species of prokaryotic recombinase.
- 13 (Cancelled) The composition of Claim 12, wherein said prokaryotic recombinase is a

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species of prokaryotic RecA protein.

14. (Cancelled) The composition of Claim 13 wherein said RecA protein species is *E. coli* RecA.
15. (Cancelled) The composition of claim 1, wherein said recombinase is a species of eukaryotic recombinase.
16. (Cancelled) The composition of claim 15, wherein said recombinase is a Rad51 recombinase.
17. (Cancelled) The composition of claim 15, wherein said eukaryotic recombinase is a complex of recombinase proteins.
18. (Cancelled) The composition of claim 1 wherein at least one of said single stranded nucleic acids contains at least one substituent.
19. (Cancelled) The composition of claim 18 wherein said substituent is a chemical substituent.
20. (Cancelled) The composition of claim 18 wherein said substituent is a protein.
21. (Cancelled) The composition of claim 18 wherein said substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification tag moieties, and nucleic acid modification moieties.
22. (Cancelled) A composition comprising at least one recombinase and a double D-loop comprising a target nucleic acid and two substantially complementary single stranded targeting polynucleotides, each comprising:
 - a) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid and to each other; and
 - b) at least one locking sequence.
23. (Cancelled) The composition of claim 22 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of said locking sequences.
24. (Cancelled) The composition of claim 22 wherein said locking sequences form a triplex anchor.
25. (Cancelled) The composition of claim 22 wherein said locking sequences form a quadruplex anchor.

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26. (Cancelled) The composition of claim 22 wherein said locking sequences form a Z-DNA anchor.
28. (Cancelled) The composition of claim 22 wherein said locking sequences form an A-DNA anchor.
29. (Cancelled) The composition of claim 22 wherein said locking sequences comprise RNA.
30. (Cancelled) The composition of claim 22 wherein said locking sequences comprise DNA.
31. (Cancelled) The composition of claim 22 wherein at least one of said targeting polynucleotides comprises a peptide nucleic acid.
32. (Cancelled) The composition of claim 22 wherein said locking sequences comprise DNA and RNA.
33. (Cancelled) The composition of claim 22, wherein said recombinase is a species of prokaryotic recombinase.
34. (Cancelled) The composition of Claim 33, wherein said prokaryotic recombinase is a species of prokaryotic RecA protein.
35. (Cancelled) The composition of Claim 34, wherein said prokaryotic RecA protein is *E. coli* RecA.
36. (Cancelled) The composition of claim 22, wherein said recombinase is a species of eukaryotic recombinase.
37. (Cancelled) The composition of claim 36, wherein said recombinase is a Rad51 recombinase.
38. (Cancelled) The composition of claim 36, wherein said eukaryotic recombinase is a complex of recombinase proteins.
39. (Cancelled) The composition of claim 22 wherein at least one of said single stranded nucleic acids comprises at least one substituent.
40. (Cancelled) The composition of claim 39 wherein said substituent is a chemical substituent.

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41. (Cancelled) The composition of claim 39 wherein said substituent is a protein.
42. (Cancelled) The composition of claim 40 wherein said substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification tag moieties, and nucleic acid modification moieties.
43. (Amended) A composition comprising at least one recombinase and a double D-loop comprising a target nucleic acid and a single stranded targeting polynucleotide comprising a first homology clamp that is substantially [corresponds] complementary to a preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and at least one locking sequence.
113. (New) The composition of claim 43 further comprising a second single stranded polynucleotide comprising a first homology clamp that is substantially complementary to said preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and at least one locking sequence.
44. (Amended) The composition of claim [43] 113 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of forms a lock structure with said locking sequence.
45. (Amended) The composition of claim [43] 113 wherein said locking sequences form an anchor structure from the group consisting of a triplex anchor, a quadruplex anchor, a Z-DNA anchor, and an A-DNA anchor.
46. (Cancelled) The composition of claim 43 wherein said locking sequences form a quadruplex anchor.
47. (Cancelled) The composition of claim 43 wherein said locking sequences form a Z-DNA anchor.
49. (Cancelled) The composition of claim 43 wherein said locking sequences form an A-DNA anchor.
50. (Cancelled) The composition of claim 43 wherein said locking sequences comprise RNA.
51. (Cancelled) The composition of claim 43 wherein said locking sequences comprise DNA.

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52. (Amended) The composition of claim [43] 113 wherein at least one of said targeting polynucleotides comprises a peptide nucleic acid.
53. (Amended) The composition of claim [43] 113 wherein said locking sequences comprise DNA and RNA.
54. (Amended) The composition of claim [43] 113, wherein said recombinase is a species of prokaryotic recombinase.
55. The composition of Claim 54, wherein said prokaryotic recombinase is a species of prokaryotic RecA protein.
56. The composition of Claim 55, wherein said RecA protein species is *E. coli* RecA.
57. The composition of claim 43, wherein said recombinase is a species of eukaryotic recombinase.
58. The composition of claim 57, wherein said recombinase is a Rad51 recombinase.
59. The composition of claim 57, wherein said eukaryotic recombinase is a complex of recombinase proteins.
60. The composition of claim 43 wherein at least one of said single stranded nucleic acids comprises at least one substituent.
61. The composition of claim 60 wherein said substituent is a chemical substituent.
62. The composition of claim 60 wherein said substituent is a protein.
63. The composition of claim 60 wherein said substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification tag moieties, and nucleic acid modification moieties.
64. A cell comprising a composition selected from claims 1, 20, or 43.
65. The cell of claim 64 which is a eukaryotic cell.
66. The cell of claim 64 which is a prokaryotic cell.
108. A kit comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each comprising
 - a) at least one homology clamp that substantially corresponds to or is substantially

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- b) complementary to a preselected target nucleic acid sequence; and
at least one locking sequence.

112. A composition comprising a double D-loop comprising a target nucleic acid and two substantially complementary single stranded targeting polynucleotides, each comprising.

- i) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence of said target nucleic acid and to each other;
- ii) at least one locking sequence; wherein said locking sequence forms a lock and a protein binds to said lock.

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Pending New and Amended Claims 43-45, 52-66, 108, and 112-113

43. (Amended) A composition comprising at least one recombinase and a double D-loop comprising a target nucleic acid and a single stranded targeting polynucleotide comprising a first homology clamp that is substantially [corresponds] complementary to a preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and at least one locking sequence.
113. (New) The composition of claim 43 further comprising a second single stranded polynucleotide comprising a first homology clamp that is substantially complementary to said preselected target nucleic acid sequence, a second homology clamp that is substantially complementary to said preselected target nucleic acid sequence, and at least one locking sequence.
44. (Amended) The composition of claim [43] 113 further comprising a secondary probe, wherein said probe is substantially complementary to at least one of forms a lock structure with said locking sequence.
45. (Amended) The composition of claim [43] 113 wherein said locking sequences form an anchor structure from the group consisting of a triplex anchor, a quadruplex anchor, a Z-DNA anchor, and an A-DNA anchor.
52. (Amended) The composition of claim [43] 113 wherein at least one of said targeting polynucleotides comprises a peptide nucleic acid.
53. (Amended) The composition of claim [43] 113 wherein said locking sequences comprise DNA and RNA.
54. (Amended) The composition of claim [43] 113, wherein said recombinase is a species of prokaryotic recombinase.
55. The composition of Claim 54, wherein said prokaryotic recombinase is a species of prokaryotic RecA protein.
56. The composition of Claim 55, wherein said RecA protein species is *E. coli* RecA.
57. The composition of claim 43, wherein said recombinase is a species of eukaryotic recombinase.
58. The composition of claim 57, wherein said recombinase is a Rad51 recombinase.

59. The composition of claim 57, wherein said eukaryotic recombinase is a complex of recombinase proteins.
60. The composition of claim 43 wherein at least one of said single stranded nucleic acids comprises at least one substituent.
61. The composition of claim 60 wherein said substituent is a chemical substituent.
62. The composition of claim 60 wherein said substituent is a protein.
63. The composition of claim 60 wherein said substituent is selected from the group consisting of intercalators, cross-linking moieties, labels, photoactive moieties, nucleic acid scission inducing moieties, purification tag moieties, and nucleic acid modification moieties.
64. A cell comprising a composition selected from claims 1, 20, or 43.
65. The cell of claim 64 which is a eukaryotic cell.
66. The cell of claim 64 which is a prokaryotic cell.
108. A kit comprising at least one recombinase and two substantially complementary single stranded targeting polynucleotides, each comprising
- a) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence; and
 - b) at least one locking sequence.
112. A composition comprising a double D-loop comprising a target nucleic acid and two substantially complementary single stranded targeting polynucleotides, each comprising.
- i) at least one homology clamp that substantially corresponds to or is substantially complementary to a preselected target nucleic acid sequence of said target nucleic acid and to each other;
 - ii) at least one locking sequence; wherein said locking sequence forms a lock and a protein binds to said lock.